Trends in
Neurosciences

Opinion

Viruses as 'Truffle Hounds': Molecular Tools for Untangling Brain Cellular Pathology

Andreas Müller-Schiffmann,[1] Svenja V. Trossbach,[1] Vishwanath R. Lingappa,[2] and Carsten Korth[1,*]

The ability of viruses to evolve several orders of magnitude faster than their host cells has enabled them to exploit host cellular machinery by selectively recruiting multiprotein complexes (MPCs) for their catalyzed assembly and replication. This hijacking may depend on alternative, 'moonlighting' functions of host proteins that deviate from their canonical functions thereby inducing cellular pathology. Here, we posit that if virus-induced cellular pathology is similar to that of other, unknown (non-viral) causes, the identification and molecular characterization of the host proteins involved in virus-mediated cellular pathology can be leveraged to decipher the non-viral disease-relevant mechanisms. We focus on how virus-induced aberrant proteostasis and protein aggregation resemble the cellular pathology of sporadic neurodegenerative diseases (NDs) and how this can be exploited for drug discovery.

Viruses Seen from Different Perspectives

The molecular basis for most chronic brain diseases including NDs remains largely unknown. Although they can be categorized by clinical symptoms and, in many cases, by neuropathological phenotypes, a number of external and internal factors such as common genetic variants, infections, immune activation, and toxic substances converge to cause clinical phenotypes by disturbing fundamental networks of cellular homeostasis in the brain. Rare familial mutations causing clinical phenotypes similar to the sporadic ones, have enabled the delineation of some major molecular pathways, but have not as yet yielded clear approaches to therapeutics for the majority of sporadic cases of NDs. Viruses may provide an orthogonal alternative.

In medicine, viruses are mainly perceived as disease-causing agents, whereas in biology their fundamental roles in ecological homeostasis [1] and as a source of evolutionary novelty are often emphasized [2]. Viruses usurp the replication mechanisms of their host cells and do not maintain their own, autonomous cellular metabolism. While it remains controversial how viruses originated [3], it is clear that viruses coevolve with their hosts [4]. Consistent with a history of virus–host cell coevolution, beneficial effects of viruses for adaptation have repeatedly been demonstrated, at both the phylogenetic and the ontogenetic level. Especially retroviruses can be seen as genetic editors that left behind numerous endogenous retroviral transcripts in the host genome, some of them of clear utility to the host. It has been estimated that 5–8% of the human genome comprises the remnants of retroviruses [5,6] and once integrated, retroviral sequences frequently shaped the host for good. One example is Arc (Arg3.1), an important regulator of synaptic plasticity [7–9], which is one of more than 100 human proteins that have been domesticated from endogenous retroviral *gag* genes [10]. Likewise, syncytin, a protein essential for mammalian placenta formation, was derived from a retroviral *env* gene [11]. Transposable elements are involved in embryonal development, pluripotency, and cell differentiation in eukaryotes [2] and some mammalian proteins are now under the control of endogenous

Highlights

Neurotropic viruses hijack host-cell proteins and modulate their physiological functions, ultimately leading to changes in proteostasis.

These host-cell proteins are often components of multiprotein complexes (MPCs) and some of them overlap with key proteins implicated in neurodegenerative diseases.

Modulation of host-cell MPCs is likely to involve the noncanonical 'moonlighting functions' of specific proteins and includes their exploitation for the catalysis of virus assembly and replication.

The fast evolutionary cycles of viruses have optimized them to exploit disease-associated weak spots in cellular homeostasis, often in ways unrelated to what can be discovered by the conventional tools of modern drug discovery.

We argue that it might be possible to harness these features of viruses to reveal molecular bases for cellular pathology and to identify novel pharmacological targets to treat neurodegenerative diseases.

[1]Department of Neuropathology, Heinrich Heine University Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany
[2]Prosetta Biosciences, 670 5th St., San Francisco, CA 94107, USA

*Correspondence:
ckorth@hhu.de (C. Korth).

retroviral long-terminal repeats, such as salivary amylase [12]. Endogenous retroviral elements have also been strongly implicated in shaping host immune responses [13].

The ultrafast generation time of viruses compared with that of their host cells has accelerated viruses' evolution and facilitated their ability to repurpose host cellular machinery to their ends. The host, in turn, is under selection pressure to counter the virus, but is impeded by the longer generation time. By virtue of their success at this evolutionary arm's race, viruses can be used to identify and interrogate weak links in molecular pathways leading to cellular pathologies in their hosts. These same weak links may become dysfunctional in taxing contexts independent of virus infection, including those involved in the pathophysiology of both genetic and sporadic NDs. Studying the interaction of viruses with their host cells may thus offer a fruitful way to reveal cellular homeostatic feedback loops that are not easily accessible using the standard molecular tools available today.

Here we advance the concept that if the cellular pathology of a non-viral disease resembles that of a virus-induced disease, the identification of specific virus-targeted **host factors** (see Glossary) can be used to decipher common molecular disease pathways. This approach seems particularly useful for the so-called 'sporadic' forms of disease (i.e., where the cause is unknown and where no obvious genetic, infectious, or toxic origins can be identified).

While in principle such a research and drug discovery strategy can be pursued for most diseases, here we focus on the cellular pathology of NDs that can be related to clear cellular phenotypes of aberrant **proteostasis** and **protein aggregation**. It is instructive to look into cancer research, where the strategy to analyze virus–host interactions to identify causes of uncontrolled cell growth and invasion has a much longer tradition and has led to key insights, and we refer readers to Box 1 for a brief discussion of this area.

Box 1. Lessons Learned from Cancer-Causing Viruses

In 2018, about 2.2 million cancer cases (13% of total cancer cases) had an obvious infectious etiology, with two-thirds owing to direct infection with oncoviruses [93]. Since the first discovery of an oncovirus by Peyton Rous in 1911 [33], seven human cancer viruses have now been identified. A hallmark of human oncoviruses is their prolonged, persistent infection in the host that can be lifelong and lead to tumor induction, which has to be viewed as collateral damage from their intracellular presence rather than a consequence of the viral life cycle. Infections with tumor viruses alone are therefore insufficient for cancer development, but add to other, non-infectious cofactors such as genetic mutations or environmental factors, which become enriched during aging and at some tipping point initiate disease. However, the direct implication of viruses in the triggering of cancers has already led to the successful development of two anticancer vaccines, targeting high-risk papillomaviruses and hepatitis B virus [94].

Mechanistically, tumor viruses impact cellular proliferation in different ways. Tumor retroviruses either directly express viral oncogenes (*v-onc*) that are derived from cellular protooncogenes (*c-onc*) (e.g., *c-myc*, *c-fos*, *c-erb*) or activate the expression of *c-onc* following integration into the host genome. Small DNA tumor viruses, such as human papillomaviruses (HPVs), polyomaviruses (e.g., SV40), and adenoviruses, encode non-structural multifunctional proteins that target tumor suppressor proteins, such as the moonlighting protein p53 or retinoblastoma protein (pRb), and drive resting cells into the S phase allowing viral replication. Remarkably, oncogenic proteins of different viral families target the same cellular proteins: HPV E6 induces the ubiquitin-mediated degradation of p53, SV40 T-antigen inhibits DNA binding of p53, and adenovirus E1B modulates the transcription-activating function of p53. Similarly, the same viruses affect physiological pRb complex formation by HPV E7, SV40 T-antigen, and adenovirus E1A [95].

Detailed studies of oncoviruses led to the identification of several additional tumor suppressor and oncoproteins that are also involved in non-virally induced tumors and therefore build the basis of our current understanding of the diverse mechanisms involved in general tumor induction [96], which also gave rise to novel drug targets in cancer therapy. Remarkably, 53 FDA-approved anticancer drugs are directed against tumor-virus-targeted host proteins [97].

Glossary

Assembly machines: transient host MPCs that catalyze biochemical reactions, including the assembly of viral capsids or other MPCs.

Autophagy: cellular process to degrade misfolded proteins, dysfunctional cellular components, bacteria, and viruses to maintain homeostasis.

Chaperones: proteins supporting the correct folding of newly synthesized proteins by protecting hydrophobic domains of the unfolded protein from inter- and intramolecular contacts with other hydrophobic domains thereby preventing protein aggregation.

Drug resin affinity chromatography (DRAC): a methodology for searching for a drug target by immobilizing an active small-molecule compound on a resin, with subsequent biochemical purification of binding protein (complexes) and proteomic identification.

Host factors: cellular proteins essential for virus replication.

Moonlighting functions: alternative roles of a protein besides its canonical, primary function. Presumably, differences in protein folding or post-translational modifications account for the distribution of subsets of the protein among the different functions.

Multiprotein complexes (MPCs): transient or stable, ordered interactions of several different proteins that by their assembly generate distinct functions.

Prion: derived from 'protein' and 'infection' and describes a protein adopting a misfolded pathological protein conformation that can be transmitted to physiological conformations of the same protein by direct contacts.

Protein aggregation: the non-physiological conglomeration of proteins into macromolecular complexes, mostly abolishing the functions of the original protein but potentially generating novel, toxic ones.

Proteostasis: protein homeostasis; the ability of a cell to maintain a balanced equilibrium of proteins.

How Can Specific Virus-Induced Host Cellular Pathology Elucidate Non-infectious Sporadic Molecular Pathogenesis with Similar Cellular Pathology?

Once a virus is internalized by binding to receptors or by capturing a suitable uptake mechanism, it typically disassembles and starts a highly orchestrated replication process, which involves a massive change in host cellular homeostasis. Depending on the nature of the virus (DNA virus, positive-strand RNA virus, or negative-strand RNA virus), replication of viral nucleic acids (NAs) can take one of two general forms. In the first, replication of viral NA exclusively uses the cell's own NA machinery, as in the case of small DNA viruses such as polyoma- and papillomaviruses. In the second, the viruses provide parts of the replication machinery, which is the case for most RNA viruses and large DNA viruses. For RNA viruses, this is done by the so-called replicase complexes, which are **MPCs** (Box 2) [14,15], illustrating the point that even the seemingly straightforward process of NA replication is performed by many proteins working together. It is noteworthy that, ultimately, host DNA replication itself is not executed by a single polymerase enzyme but by a complex 'replisome' comprising at least 50 proteins [16], supporting the notion that MPCs are responsible for viral cellular functions.

Some viruses have evolved massive strategies in their arms race with their host cells: to protect themselves from host recognition of unique virally induced molecules, such as double-stranded RNA, many viruses have evolved to form viral factories (i.e., cellular mini-organelles where most replication and assembly steps are executed in a concealed space) [17,18]. The fact that viral factories are built from host-cell components illustrates viruses' agility in recruiting host-cell proteins and reorganizing subcellular compartments, as well as in arranging membraneless organelles [19], liquid-like phase separations [20], and aggresome-like complexes shielded by a vimentin-containing cage [17]. In the case of rabies virus, such factories are even visible by light microscopy and have been termed Negri bodies. These are membraneless, liquid–liquid phase separation organelles of a few micrometers in size that can be stained by a mixture of fuchsin and methylene blue [21].

Box 2. Host-Mediated Catalysis of Viral Assembly

A body of literature suggests that viral capsid formation does not occur through spontaneous self-assembly, as had long been believed [98]. Instead increasing data support the view that capsid formation is catalyzed by host enzymes [28,99–101]. The evidence for this rests on two different kinds of experiments. First, capsid assembly has been reconstituted in a protein synthesis-dependent manner in cell-free systems and shown to be nucleotide triphosphate hydrolysis-dependent, a telltale sign of enzymatic activity [100]. The second is through functional reconstitution of capsid assembly, either by ultracentrifugal fractionation of extracts [100] or by affinity chromatography in which small molecules that modulate assembly serve as affinity ligands to fractionate an extract [28]. In either fractionation approach, the depleted extracts remain capable of capsid protein synthesis, but are no longer capable of assembly. However, assembly can be reconstituted by add-back of the removed fraction. These enzymes of assembly, which have been termed **assembly machines**, were long overlooked, perhaps because they are transient MPCs, and they could provide a novel molecular basis for homeostasis. Viruses are likely to manipulate signaling pathways [102] to alter assembly machine composition and action. The resulting aberrant assembly machines favor the needs of the virus over those of host homeostasis. The normal function of those assembly machines are critical points of regulation of host-gene expression. Since, as discussed, viruses in effect run evolution about 50 000× faster than their human hosts, it is unsurprising that they have identified some of our greatest metabolic and biochemical vulnerabilities over deep, evolutionary time – and have coevolved to take advantage of them. If assembly is as important as suggested, assembly machines should be important viral targets, and small molecules targeting allosteric sites that control assembly machine composition and action represent a novel antiviral therapeutic strategy [28,29]. It is likely that the same metabolic weak links that viruses take advantage of are the cause of non-viral diseases, in which case assembly modulation would represent an important therapeutic strategy for their treatment [49,103]. If we can convert viruses into 'truffle hounds' to identify the high-value drug targets not accessible to conventional proteomics, novel small-molecule therapies as effective at correcting disease as viruses are at causing disease could be identified (see Outstanding Questions). If the above paradigm is correct, two predictions can be made with regard to NDs: (i) there should be a correlation between particular viral families (or, more precisely, their aberrant assembly machines) and particular NDs; and (ii) the small molecules that target aberrant assembly machines should be effective against at least a subset of the non-viral causes of that ND. For both of those predictions, empirical data have recently been reported [57,77,104].

During the recruitment of host proteins, almost all subcellular compartments are affected in a virus (family)-specific way. These effects include nuclear pathology and protein secretory- or degradation-pathway deficits [22], all contributing to the subsequent inability of a cell to maintain protein homeostasis, or 'proteostasis'. Loss of proteostasis is a common feature of cells stressed by toxins, starvation, infection, or other causes that can lead (among other effects) to the accumulation of protein aggregates. Under physiological conditions, proteostasis is controlled by stable networks of macro-MPCs involved in protein synthesis (ribosomes), correction of protein folding (molecular **chaperones**), or targeted degradation of nonfunctional proteins (autophagosomal/ lysosomal or proteasomal pathways; for a review see [23]).

Cellular chaperones are involved in virtually every major step of the viral life cycle. They facilitate the internalization of the virus, destabilize the viral capsid to release the viral genome, stimulate translation, guide co-translational folding to prevent aggregation and proteasomal degradation, support the nuclear import of viral proteins, and finally assist capsid assembly [24].

Autophagy is a process contributing to the maintenance of cellular homeostasis. Autophagy encompasses three major routes: (i) the direct uptake of cellular material into lysosomes (microautophagy); (ii) lysosomal associated membrane protein 2A (LAMP2A)-dependent uptake of misfolded proteins via chaperone-mediated autophagy; and (iii) macroautophagy that allows the sequestration of protein aggregates and damaged organelles that are engulfed by a double-membrane vesicle called the autophagosome, which later fuses with a lysosome to form the autophagolysosome [23]. Upon infections with pathogens, autophagy is activated to target viral components for degradation. Autophagy, therefore, serves as part of the cellular innate immune defense, a process termed xenophagy [25]. However, viruses evolved to adapt to these processes and acquired mechanisms to hijack and subvert autophagy for their own benefit. Several RNA viruses such as poliovirus use double-membrane autophagosome-like vesicles for replication and use them for the non-lytic release of matured virions from the cell, while blocking their maturation to destructive autophagolysosomes [26].

How do viruses induce such massive repurposing of host-cell proteins? Many host proteins perform so called 'moonlighting' functions, which are additional biological functions besides the proteins' canonical roles [27]. Viruses seem to be particularly good at 'divining' the moonlighting potential of host proteins and select proteins having **moonlighting functions** as targets for exploitation. By orchestrating the recruitment of host proteins, viruses can cause proteins previously not associated with one another to form novel, transient MPCs, which can either remain transient or amount to persistent viral factories [17]. It is unknown how exactly viruses recruit host proteins by their moonlighting functions, but one may speculate that structural information in viral proteins provides interaction sites to one of the host proteins, or an ensemble of them, via allosteric sites. These MPCs include those shown to catalyze the assembly of capsids [28,29]. Weak interactions of multiple proteins can result in a novel catalytic activity [30] and such complexes are eventually assembled co-translationally [31] (Box 3). Alternatively, host signaling pathway manipulation, for which viruses are notorious, could achieve the same ends more indirectly.

One can also envision scenarios where a specific virus accelerates a specific cellular pathology that can occur also in the absence of virus. We would argue that, in these situations, identifying the host proteins targeted by the virus may provide clues towards causative molecular mechanisms relevant to the pathology more generally (Figure 1, Key Figure). In this sense, viruses can be used as 'truffle hounds' to 'scent' (i.e., identify) the cellular weak links in the form of moonlighting host proteins common to both the virus-induced and their virus-independent counterparts.

Box 3. Why Are Transient MPCs Difficult to Detect?

Scientific tools, methods, and protocols are developed in a historical context, based on what they are needed for, which in turn is based on the prevailing scientific paradigm, which is likewise constrained by the technological limitations of the place and time. In this regard, two very different problems have conspired to make transient MPCs, such as those recruited by viruses and that are disease relevant in other contexts, difficult to detect. Proteomic methods were informed originally by the 'one gene, one enzyme' hypothesis and therefore initially pursued individual gene products as targets, to manipulate gene expression. However, there has been a growing appreciation that, in living cells, proteins do not function just individually, but rather, often, in concert with other proteins as MPCs [105,106]. How do MPCs come together correctly given the crowded environment in the cytoplasm [107–109]? What has eluded detection by the conventional tools of today is the catalytic machinery that allows the thermodynamics of assembly to occur on a physiologically relevant timescale. A second reason why transient MPCs have been overlooked to date is that, despite a growing body of literature that many if not most proteins are multifunctional [27], many scientists today persist in the expectation that measuring amounts of particular proteins is sufficient to assess their role in health and disease. However, if a given protein has different functions, the subset involved in function A might be up- or downregulated without affecting the subset involved in function B, C, or D. In this context, the signal-to-noise challenge for the detection of a change in the level of the relevant subset A becomes potentially daunting, especially if the number of functional forms is large or the subset of interest represents a small percentage of the total amount of that protein, as has been demonstrated for viral assembly [28,110]. It is difficult to appreciate what is difficult to detect, and what one cannot detect is generally not looked for, and when found inadvertently is easily misinterpreted. The development of new tools, including cell-free protein synthesis and assembly (CFPSA) systems [32,111] and novel methods of **drug resin affinity chromatography (DRAC)** [28,112] has made possible the detection of a new realm of more physiologically relevant drug targets. We would argue that application of the viral 'truffle hounds' paradigm provides an efficient path from the new targets to new drugs, even before completion of the paradigm shift [113–115].

Compared with mutation-driven disease mechanism analysis, viral infections allow functional assays on the cellular *in vitro* as well as the *in vivo* level, thus facilitating drug discovery approaches by valid bioassays. Virus-guided drug discovery for 'concomitant' sporadic diseases may also take advantage of reversing the traditional drug discovery workflow and ultimately accelerate the process by first identifying potent drugs, and only then their molecular targets (Figure 2). Along these lines, cell-free protein synthesis and assembly (CFPSA) systems have been combined for the study of viral assembly, to establish a novel drug discovery screen platform [28,32] (Box 3).

How the Specific Use of Viruses Could Complement Research Strategies in NDs

In neuroscience, the possible molecular roles of viruses in disease pathogenesis have been less well investigated compared with, for example, cancer research, a field of research where the involvement of viruses in the disinhibition of cell growth has been recognized for a long time [33] (Box 1). It is not our intention to suggest that specific viruses are a major cause of NDs, although we do not exclude the possibility that, in a subset of cases, viruses could trigger cascades of cellular events that may culminate in ND. Rather, we propose that changes in cells, which are induced by viruses and resemble the cellular pathology of sporadic NDs, can accelerate the discovery of the causes of these diseases through the identification of moonlighting proteins with dual roles in viral replication and proteostasis. In the following, we briefly review current concepts of neurodegeneration, but due to space limitations this overview is incomplete and it intentionally focuses on specific aspects of the topic. Fundamentally, we see the approach proposed in this Opinion article as complementary to other concepts rather than an exclusive path to address NDs. The clear advantage, we would argue, of identifying moonlighting proteins through viral truffle hounds is that these findings may directly lead to novel and specific drug targets (Figure 1 and Box 3).

NDs such as Alzheimer's disease (AD), frontotemporal dementia (FTD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) have historically been defined by specific clinical symptoms accompanied by the deposition of specific protein aggregates in the brain. In AD, the aggregates comprise extracellular Aβ and intracellular hyperphosphorylated tau, whereas in FTD mutant, aggregated tau is present. In PD, α-synuclein containing Lewy bodies are detected in

Key Figure

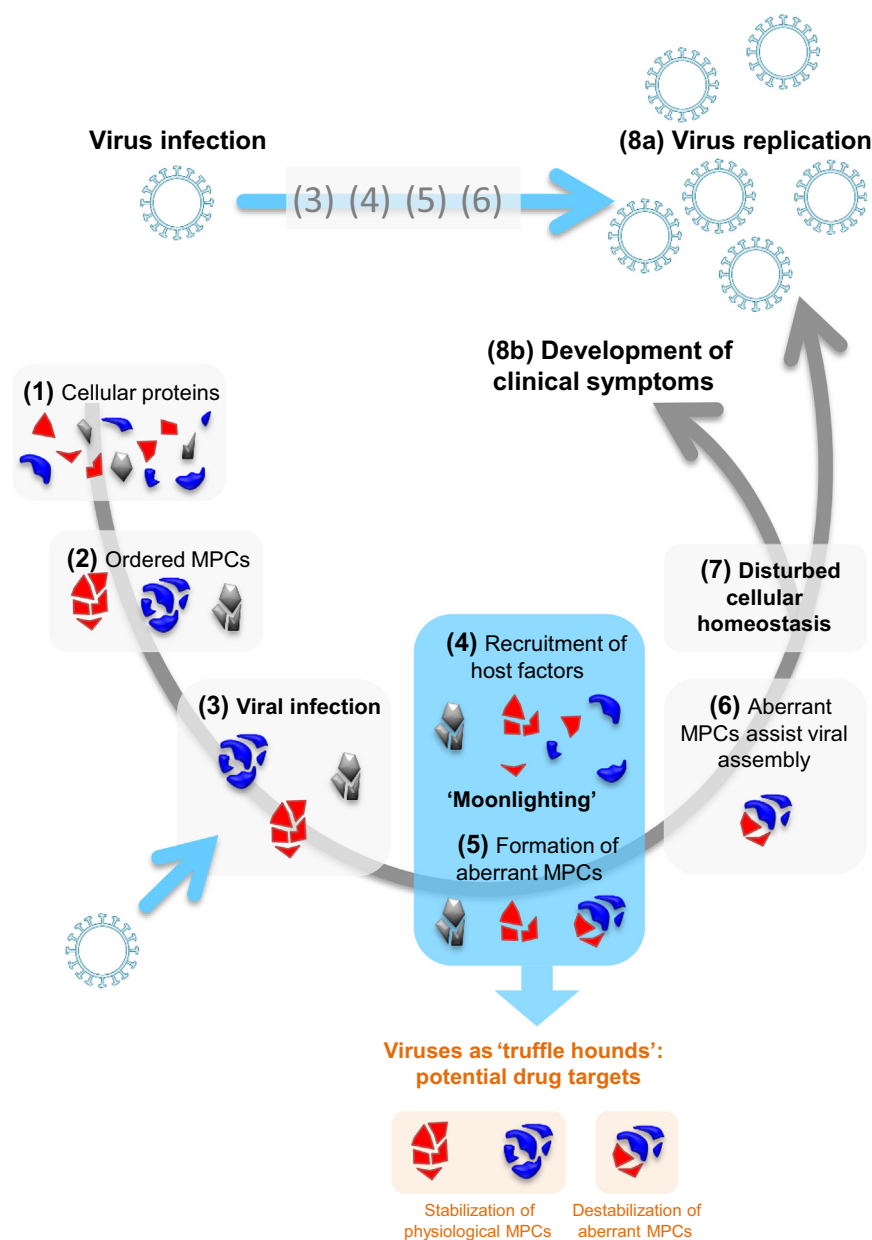## Virus Replication and Assembly Is Assisted by Host Factors

Figure 1. Cellular host proteins (1) can assemble into physiologically interacting protein networks or multiprotein complexes (MPCs) (2). On viral infection (3), some of those physiological homeostatic interactions are disturbed through the recruitment (4) and reassembly of host proteins into novel MPCs (5) assisting virus replication and assembly (6). Disturbed cellular homeostasis through massive recruitment of host proteins (7), while ensuring successful virus replication (8a), may lead to cellular pathology and, ultimately, clinical symptoms (8b). Both, the stabilization of physiological MPCs and the destabilization of aberrant MPCs may provide novel pharmacological targets for antiviral activity and for the prevention of cellular pathology.

the dopaminergic cell bodies of the substantia nigra. In FTD and ALS, TAR DNA-binding protein 43 (TDP-43) has been found aggregated in stress granules, and other aggregated proteins also play roles [34–36]. However, we now understand that in many cases no aggregates are exclusive markers, since, for instance, Aβ plaques have also been found in PD, α-synuclein precipitates in AD, and aggregated TDP-43 in FTD [37], which hints at a continuous disturbance of proteostasis (i.e., an imbalance between the generation of misfolded proteins and their degradation). In addition to external factors, these changes are likely to be induced by polygenic risk factors, a growing number of which have been identified by large genome-wide association studies (GWASs). These studies also revealed the pleiotropic character of some of the identified gene loci, meaning that a specific gene locus is associated with several NDs (and sometimes also non-degenerative ones; for a review see [38]). A number of the risk genes are expressed by microglia. However, it is currently under debate whether activated microglia protect against ND by clearance of protein aggregates or whether they are harmful to neurons by secreting inflammatory factors with neurotoxic potential [39,40]. Microglia as the major cells activated in neuroinflammation are also an interface between systemic immune activation, including from viruses. Their activation during an antiviral systemic immune response can be detrimental to neurons and thus provides an indirect route for how viruses can harm the brain [41]. Furthermore, cells of the adaptive immune system have also been demonstrated to have roles in mechanisms of neurodegeneration [42,43]. This Opinion article, however, is concerned only with the direct effects of viruses on cells (i.e., after cell entry) for their use as identification tools.

Based on the clinical and neuropathological symptoms of rare familial cases and the majority of sporadic cases of NDs (but with an earlier disease onset), cellular and animal models expressing familial mutations have been widely used to investigate ND pathways. This has led far but also raised questions. For example, antibody-based therapy against fibrillar Aβ has been developed and validated in genetic animal models of AD but failed in human trials with sporadic AD cases [44].

For some proteins, once protein aggregates have emerged, they can propagate and spread in a **prion**-like manner by seeded nucleation (i.e., the conversion of previously non-aggregated forms of those proteins to aggregated conformers; for a review see [45]). The paradigmatic examples are the spongiform encephalopathies, such as Creutzfeldt–Jakob disease. These fatal NDs were initially thought to be caused by viruses [46], but it was ultimately shown that they were initiated solely by a disease-associated conformer of the endogenous prion protein (PrP) [47,48]. Whereas the propagation of existing prions is well established, it is currently unknown how the initial *de novo* prions originate. Work from our group has shown that exogenous stressors, including viral infections, could specifically lead to disturbances in proteostasis and trigger such 'first prions' [49]. Such hits may occur multiple times independently and accumulate through both viral and non-viral origins, increasing aberrant proteostasis. The potential relevance of prion-like propagation of nucleating proteins in NDs is that virus-induced aberrant proteostasis can trigger a cascade of cellular events where, later, the original impact can no longer be determined, as in a 'hit-and-run' crime. Similar ideas have been advanced for somatic mutations, which may also provide critical seeds triggering prion-like replication of protein aggregation in NDs [50], and it is conceivable that virus-induced proteostasis and somatic mutations may act in concert.

All of the abovementioned concepts assume that the aggregates themselves are the starting points of NDs. An alternative, allegorical, and non-mutually exclusive view of the situation of accumulating protein aggregates is that the aggregates are analogous to the wreckage after a car crash at an unmarked intersection. As a means of preventing future car crashes, placement of a stop sign is more appropriate and effective than simply calling more tow trucks. Antibodies and other
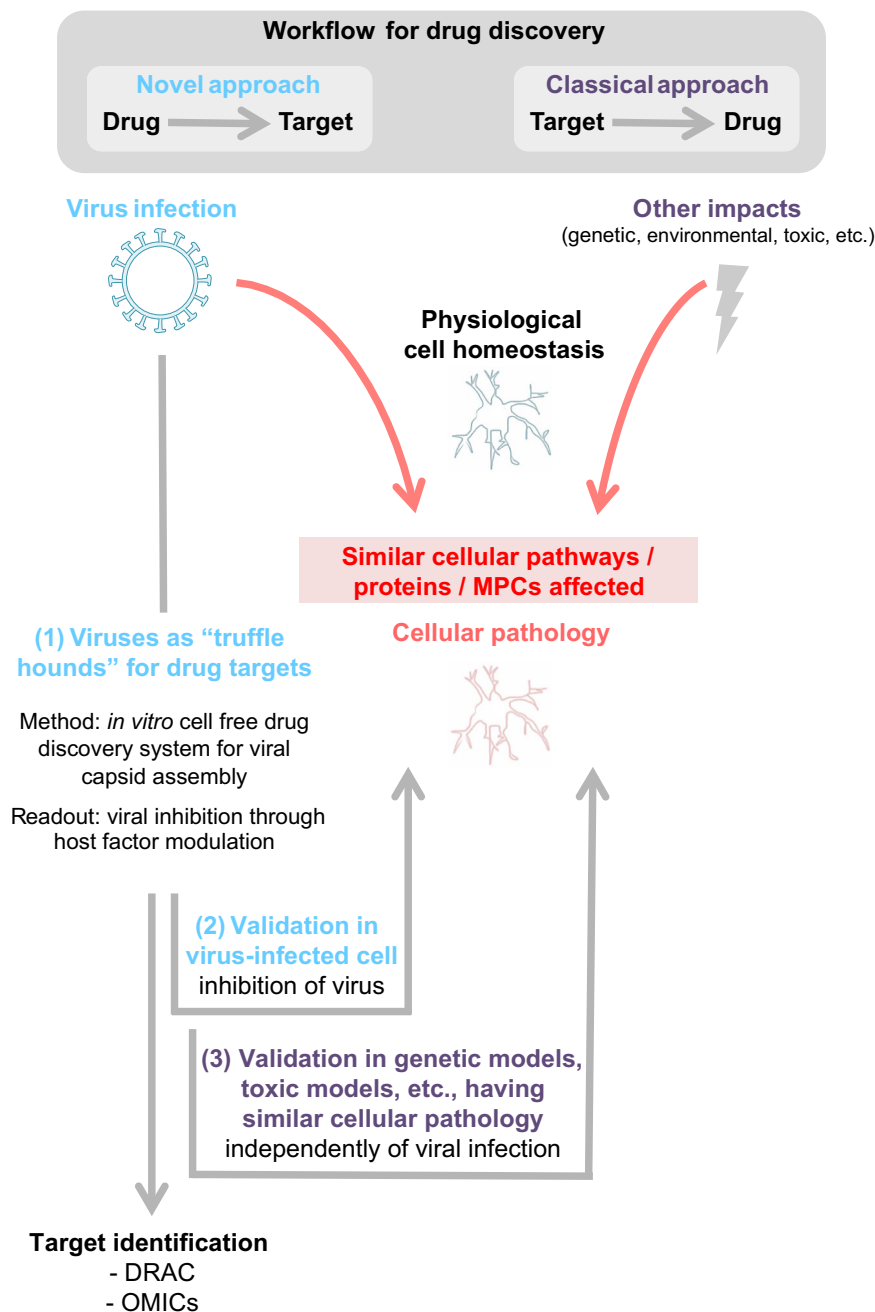
Figure 2. Drugs Before Targets: Reversal of the Traditional Drug Development Scheme. Drugs against viral capsid assembly targeting cellular host factors can be screened in cell-free *in vitro* systems [28]. Once validated for antiviral activity by appropriate cellular assays, targets can be determined using, for instance, drug resin affinity chromatography (DRAC). If a cellular pathology similar to that elicited by viral infection can also be caused by a genetic or toxic lesion, host factors similar to those recruited during viral infection may be affected. Hence, drugs targeting those host factors and identified through an antivirus screen may become lead compounds for correction of the genetic or toxic lesion.

therapies targeting the aggregates are simply cleaning up the wreckage, but not preventing similar future car crashes. Returning to the cellular pathology of NDs, host-targeted therapeutics that address the aberrant repurposing of the host machinery of an upstream target are more analogous to the stop sign analogy above. While certainly the neurotoxicity of aggregates and that of potentially elicited prions has been demonstrated (i.e., do not leave the wreckage on the street since more cars may crash into it), if upstream factors continue to lead to novel, independent car crashes it is also important to install stop signs to prevent those crashes in the first place. The equivalent of installing a stop sign is then to correct an aberrant protein assembly that impairs proteostasis and that continuously leads to new wreckage in the form of protein aggregates.

Such MPC modulation offers then a novel therapeutic approach for several reasons. First, it has been massively exploited by viruses: assembly is an essential step bridging replication and propagation, the *sine qua non* features of viral infection. Second, it is an indirect means of addressing the problem that mimics how biological regulatory feedback loops operate by adjusting to compensate (Figures 1 and 2 and Boxes 2 and 3).

Virus Interactions with Cells of the Central Nervous System (CNS)

In the clinical neurosciences, a molecular role for viruses in disease pathogenesis requires further investigation. The blood–brain barrier (BBB) and the peripheral immune system normally protect the CNS from invasive pathogens, including viruses. However, some viruses, likely through their coevolution with the hosts, have developed multiple mechanisms to invade the CNS, including: (i) using penetrating immune cells as 'Trojan horses'; (ii) inducing permeability-inducing genes; and (iii) spreading along the olfactory tract, where olfactory neurons bridge between the outside environment and the brain, thereby bypassing the BBB, for topical invasion [51,52].

The following sections review empirical evidence associating viral infections with the cellular pathology of NDs. The goal is to demonstrate that a very specific ND-associated cellular pathology rather than general, unspecific effects have been reported for each ND by distinct viral families, which makes indirect effects (e.g., immune activation) less likely as explanations. These specific, emerging associations are not thought to provide a sole causal explanation for these NDs, but they do offer a hint, we would argue, about candidate virus families, which can be used as tools for further investigation of overlapping, dysregulated host proteins in the corresponding NDs.

Influenza and PD

Influenza viruses have consistently been reported to be associated with brain diseases and influenza infections can directly lead to encephalitis. Reports linking influenza infections to post-infection Parkinsonism based on the wave of post-encephalic Parkinsonism following the 1918 flu pandemic (1918–1920) have been controversial [53,54]. However, mechanistic experiments in mice infected with influenza virus have repeatedly demonstrated a causal relationship between influenza infection and α-synuclein-related cellular pathology: mice displayed loss of dopaminergic neurons in the substantia nigra [55], the infection directly induced α-synuclein protein aggregation in brain areas co-staining for the viral antigens [49], and caused narcolepsy-like symptoms [52]. Mechanistically, influenza virus infection disrupts autophagic flux in neuronal cells by interfering with both the formation of autophagosomes and their fusion with lysosomes [49], but other ways of interfering with proteostasis have been reported as well [56]. Repeated viral infections together with genetic or other environmental risk factors – for example, a synergistic effect of influenza infection and the dopaminergic-neuron-specific toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) –

accelerated the loss of dopaminergic neurons [57]. Disturbances of cellular autophagy by influenza virus infection can induce the genesis of a critical mass of misfolded proteins (first prions) that then could contribute to the triggering of molecular processes leading to PD [49]. A general rapamycin-induced upregulation of autophagy has been shown to decrease the toxic accumulation of α-synuclein [58].

Herpesviruses and AD

Herpesviridae, such as herpes simplex virus 1 (HSV-1), were associated with AD decades ago and it is currently debated whether human herpesvirus 6A and 7a might contribute to AD [59–61]. While a general connection between herpesvirus infection and AD remains elusive, a retrospective cohort study found an increased risk for dementia in the herpes-infected patients that was attenuated when drugs against herpesvirus were taken [62] and another study reported increased anti-HSV-1 IgM antibodies as evidence for an activated HSV-1 infection [63]. Remarkably, apolipoprotein E4 (ApoE4), the strongest common genetic risk factor for the development of senile AD, also increases brain infiltration by herpesviruses [64]. Further, brain areas that tend to be affected early in AD, such as the entorhinal cortex, hippocampus, and amygdala, are also ones that are prominently affected by herpes encephalitis [65].

Herpesviruses can also directly affect Aβ pathology. The HSV-1 glycoprotein B has a high sequence homology with the Aβ peptide and has been shown to act as a seed for Aβ aggregation [66]. It was observed that Aβ oligomers could bind to herpesvirus surface glycoproteins accelerating the deposition of Aβ around the virus *in vitro* and *in vivo*. Along these lines, a role of Aβ as part of the innate immune system with antimicrobial functions has been proposed [67]. This could be a general function of amyloidogenic proteins, since a recent study found that amyloids can be engineered with specific antiviral activity [68]. In addition to the direct effects of HSV-1 on Aβ, herpesviruses interfere with proteostasis by impairing autophagy during acute/primary infection or reactivation from latency at various stages [69]. Gamma-herpesviruses block late steps in autophagy by hijacking autophagosomes facilitating transport of the mature viruses to the plasma membrane. Other herpesviruses, such as HSV-1, dysregulate autophagy in infected neurons by directly binding and inhibiting Beclin-1, an essential autophagy factor [70]. Independent from a potential viral influence, autophagy is also disrupted in sporadic and familial AD at various steps of the autophagic process: initiation of autophagy, transport of the autophagosome, and fusion of the autophagosome with the lysosome. Therefore, modulation of autophagy is currently being considered as a potential treatment option in AD [71].

Retroviruses and ALS

TDP-43 and FUS are proteins both discovered to be repressors of HIV-1 transcription and both have also been found to be involved in a form of protein aggregation pathognomonic of ALS [35,72–74]. In ALS, TDP-43 is mislocalized to the cytoplasm [75] and this stress-induced aggregation of TDP-43 is discussed as a cause of motor neuron death in ALS [76]. Known human ALS mutations in both of these proteins expressed in model organisms as transgenes cause ALS-relevant phenotypes. Human endogenous retroviruses have been associated with ALS pathogenesis. Environmental factors can trigger the expression of retroviral transcripts, which have been observed in samples from ALS patients [77,78]. In addition, enteroviral and encephalomyelitis infections have been shown to trigger aggregation of TDP-43 in mouse models [79,80]. By analogy, aggregation of FUS has also recently been shown to be initiated on viral infections [81]. Thus, it is tempting to speculate that a MPC coopted by viruses may be involved in ALS pathogenesis.

Novel Coronaviruses

The current SARS-CoV-2 pandemic is still too new for definite statements to be made about long-term neurological consequences; however, coronaviruses can clearly be neurotropic [82–85]. Many COVID-19 patients suffer acutely from anosmia [86–88], indicating that olfactory neurons might be affected by SARS-CoV-2, and it seems plausible that the olfactory nerve might be an entry route for the virus into the brain. Neurological and psychiatric symptoms in COVID-19 patients are manifold [86–89]. In a community cohort of COVID-19 patients (n = 509), neurological manifestations were present in about 82% of the patients during some point along the disease course and about a third of the patients developed signs of encephalopathy that were correlated with increased mortality [90]. In another study of 43 *post mortem* brains from COVID-19-deceased patients, neuroimmune activation was demonstrated in many of the samples, but no encephalitis [91]. Intriguingly, for the SARS virus from the first epidemic in 2003, it has been reported that the virus can silently use the CNS as a reservoir [82,83]. Our group has recently shown that SARS-CoV-2 easily infects brain organoids and targets neurons, where it induces tau mislocalization and hyperphosphorylation [85]. It remains to be shown whether these potential effects on neurons are reversible or may have eventual long-term effects and trigger chronic brain disease as outlined in the previous text. In a recent review on all reported SARS-virus-infected patients, apathy and chronic fatigue where found as the most frequent long-term neurological or psychiatric symptoms in the aftermath of acute infection [89]. Anecdotal reports of probable cases of PD after COVID-19 [92] add to the list of possible long-term effects of SARS-CoV-2, justifying diligent monitoring of the situation. Importantly, as for other viral diseases, it has to be carefully dissected whether such chronic symptoms are due to residual peripheral immune activation, increased cytokine levels, microglial activation, direct effects of the virus on neurons – as broached here – or any combinations thereof.

Concluding Remarks and Future Perspectives

In the previous sections, we discussed how virus-induced aberrant proteostasis and protein aggregation can resemble the cellular pathology of sporadic NDs, including by the recruitment of common host proteins using their moonlighting functions. We have given a current overview on which viral families are associated with which cellular pathologies characteristic for each ND, although the picture outlined here may not be exhaustive. Over deep evolutionary time involving a tight arms race with their host cells and everchanging selection pressures, and by virtue of their extremely short generation time, those viruses associated with ND-typical cellular pathology obviously have an edge over their host cells.

Can one develop drug screens that take advantage of what viruses have learned (see Outstanding Questions)? We would argue that such an approach could lead to the discovery of new targets and mechanisms, some of which may be crucial to a deeper understanding of the pathophysiology of brain diseases (Figure 2). Put another way, viruses themselves might be used as experimental tools – truffle hounds – to dissect disrupted cellular homeostasis when a cellular phenotype of a viral infection resembles one or another cause (e.g., a genetic lesion, an environmental toxin, a developmental disorder). Viruses have the advantage that their journey through the host cells follows discrete and sequential interactions with host proteins, contrary to, for example, oxidative stress paradigms where changes in too many processes occur simultaneously, possibly randomly, and in a non-programmed fashion, making them difficult to sort out. The identification of relevant host cell factors, including, for example, transient MPCs as cross-sections between viral, genetic, and toxic causes, may advance our understanding of the most common sporadic forms of ND. This approach may also make possible the development of new drugs that prevent the aberrant assembly likely to be involved in ND pathogenesis. The execution of such an approach, however, will require a revision of conventional drug discovery thinking in a number of fundamental respects.

In particular, the focus on individual proteins and their active sites may be too shortsighted and give away effective alternative options. A key challenge, then, is to develop the tools and methods to probe for allosteric sites on MPCs that would target the interactions of several proteins rather than only one. The viruses can teach us how.

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